PROGRESS REPORT

June 1, 1991

"Bioorganic Models for Protein Ion Channels"
(Contract number N00014-90-C-0083, R&T CODE 4412065)

Principal Investigator: W. F. DeGrado
Du Pont de Nemours (E.I.) and Co., Wilmington, DE.
Contract Title: Bioorganic Models for Protein Ion Channels

Start Date: April 1, 1990

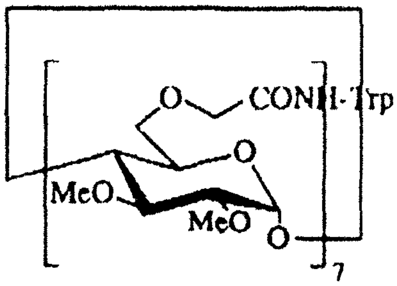
Research objective: To investigate the structural features responsible for the conduction of ions through ion channel proteins; to design metal-binding peptides; to develop novel biosensing devices.

Progress (year 1):

Spectroscopic Studies We have prepared seven variants of the peptide, H_2N-(LeuSerSerLeuLeuSerLeu)$_3$.$CONH_2$ in which a residue in the central heptad has been replaced by Trp. Extensive fluorescence measurement indicate that the peptide binds to phospholipid (PL) vesicles (PL/peptide ratio 100:1, no transmembrane voltage) with its helical axis parallel to the surface of the membrane. Trp residues that replace Leu are efficiently quenched by phospholipids bearing a nitroxide at various positions along their fatty acid chains, while Trp residues that replace Ser are efficiently quenched by aqueous-phase quenchers. These findings indicate that the voltage-dependence of channel opening in planar bilayers involves a voltage-induced change in peptide orientation from a surface to a transbilayer configuration.

Modulating channel lifetime We have increased the mean open time of our peptide channels by designing longer, helical peptides whose interaction surface extends beyond the hydrophobic side of the bilayer. Thus, adding the heptapeptide sequence, Tyr-Ala-Ser-Asp-Arg-Ser-Leu to the N-terminus of (Leu-Ser-Ser-Leu-Leu-Ser-Leu) increased the channel lifetime from approximately 5 msec to approximately 1 sec.

Template-Assembeled Channels Toward our goal of designing channel-forming helix-bundles of defined size the peptide (LSSLLSL)3, and derivatives thereof, were reacted with β-cyclodextrin **1**. The tryptophan residue of cyclodextrin **1** served as a chromophore and thus facilitated the monitoring of the coupling reaction. The isolation of the product was in each case hampered by solubility problems. We found that the introduction of α-aminoisobutyric acid (B) into the peptide, (LSLBLSL)3, greatly improved the solubility properties of the reaction mixture. The results are very encouraging and we are currently isolating the product to prove the structure and evaluate the properties of the assembly. We also attached the (LSLBLSL)$_3$-peptide to a tetraphenylporphyrin derivative (provided by Dr. Groves et al., Princeton University) to make a tetrameric α-helix assembly. The isolated bundle shows significant α-helix content when inserted into vesicles and preliminary data indicates that it is capable of passing protons across phospholipid bilayers. The next goal will be to do specific amino acid substitutions in the peptide, attach it to the template, and evaluate the effects of the amino acid side chain on ion conductance and selectivity.



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Biosensing devices. Two biotinylated ion channel peptides have been synthesized:

Biotin--Linker--(LeuSerSerLeuLeuSerLeu)$_3$-CONH$_2$
Biotin--Linker--Lys-Glu-Glu-Gly-Gly-Pro-Leu-(LeuSerSer LeuLeuSerLeu)$_3$-CONH$_2$

The first consists of a model ion channel peptide discussed previously, with biotin attached via one of two commercially available linkers (Pierce Chemical Company) -- either a pentanoyl or an ethyl 1,3'-dithiopropionate grouping. The second peptide contains a long, hydrophilic tether that should help hold the biotin group at some distance from the membrane. Both peptides form channels in planar bilayers. No major change in conductance properties were determined when avidin was added, even though it could be shown that avidin bound the biotin. In related work, a copper-binding peptide (His-Gly-Gly) was added in place of biotin to the peptides. Again, no major change in conductance was observed when Cu^{2+} was added. Therefore, we have abandoned work on this subject.

De Novo Design of Metal Binding Sites in Proteins In previous work, we designed a Zn^{2+}-binding site in a water-soluble four-helix bundle protein by introducing two His residues on one helix and a third His on a linked helix, such that the ligating atoms could occupy three positions of a tetrahedron or iscosahedron. In this period we have studied the folding properties of the designed protein. In the absence of metals, the protein shows an

NMR spectrum consistent with a "molten globule" structure, showing a high helical content but little dispersion in the resonances of the side chains. In addition, the protein binds the hydrophobic fluorescent probe ANS. Upon addition of metal ions, the protein becomes considerably more ordered as assessed by a dramatic increase in the dispersion of its NMR spectrum. We wish to begin structural determination of the metal-bound form of the protein by NMR methods in the next period. We have also investigated the binding specificity of the protein for various metals and find $Zn^{2+} > Co^{2+} > Cu^{2+} > Mg^{2+} > Ca^{2+}$. Finally, we have extended our original design to include a 2-His,1-Glu Zn^{2+}-binding protein.

We have also begun work on the design of a 2-His, 1-Cys copper-binding protein intended to mimic the properties of blue copper proteins. The protein has been constructed and shown to bind metal ions including copper. Phy^ical and spectroscopic analysis will be carried out in the next year.

Significance The design of ion channel peptides should lead to a better understanding of the mechanism of natural ion channel proteins, and may also lead to the development of elements to be used in biosensing devices. In addition, our design of a metal-binding protein should help decipher the mechanisms of cation selectivity in natural systems, and may be elaborated into sensing devices.

Work Plan (year 2): The objectives of year 2 are several-fold: to better characterize the properties of our designed peptide channels, design of template-assembled channels, design new metal-binding proteins, and characterize the conformational properties of metal-binding proteins. In this period of the grant, a large portion of the work will be directed at computer graphics (for design efforts), synthesis and NMR structure determination.

Inventions (Year 1): None

Publications and Reports

1. Handel, T. and DeGrado, W. F. "De Novo Design of a Zn^{2+}-Binding Protein" *J. Amer. Chem. Soc.* **112**, 6710 (1990).
2. Osterhaut, J., J., Jr., Handel, T., Na, G., Toumadje, A., Long, R. C., Connolly, P. J., Hoch, J. C. Johnson, W. C., Jr., Live, D., DeGrado, W. F. "Characterization of a Peptide Designed to Form a Four-Helix Bundle" in press in *J. Amer. Chem. Soc.*
3. Chung, L., Lear, J. D., and DeGrado, W. F. "Fluorescence Studies of the Secondary Structure and Orientation of a Model Ion Channel Peptide in Phospholipid Vesicles" submitted to *Biochem.*

Presentations (Invited lectures, April 1-June 30)

William F. DeGrado: Bristol-Meyer Squibb, U. of Colorado, U. of Chicago, Rockefeller University, French-American Chemical Society, U. of Pennsylvania, AT&T Bell Labs, ACS Medicinal Chemistry Meeting.

Tracy Handel: U. of Chicago, U. of Mich., Berkeley, UCLA, Johns Hopkins, Keystone Meeting on Protein Structure, Proteins Gordon Conference, Office of Naval Research Conference on Bioorganic Chemistry, Stanford, Biochem. Dept., ASMB/AAI meeting, U. of Tennessee, ACS meeting (Atlanta), Caltech, Lehigh University.

ANNUAL REPORT QUESTIONAIRE

Principal Investigator	William F. DeGrado
Institute	DuPont Merck Pharmaceutical Company Biotechnology Department
Grant title:	Bioorganic models for ion channel peptides
Period of performance	June 30, 1990 - June 1, 1991
Number of publications	3
Patents/inventions	
Number of trainees	3
female	3
Minority	1 Asian
Not U.S citizens	1
Awards, Honors	
Equipment Purchased	None
Email	DEGRADO%ESVAX%DUPONT.COM@RELAY.CS.NET

DE NOVO DESIGN OF

ION CHANNEL PEPTIDES & METAL ION BINDING PROTEINS

Objectives

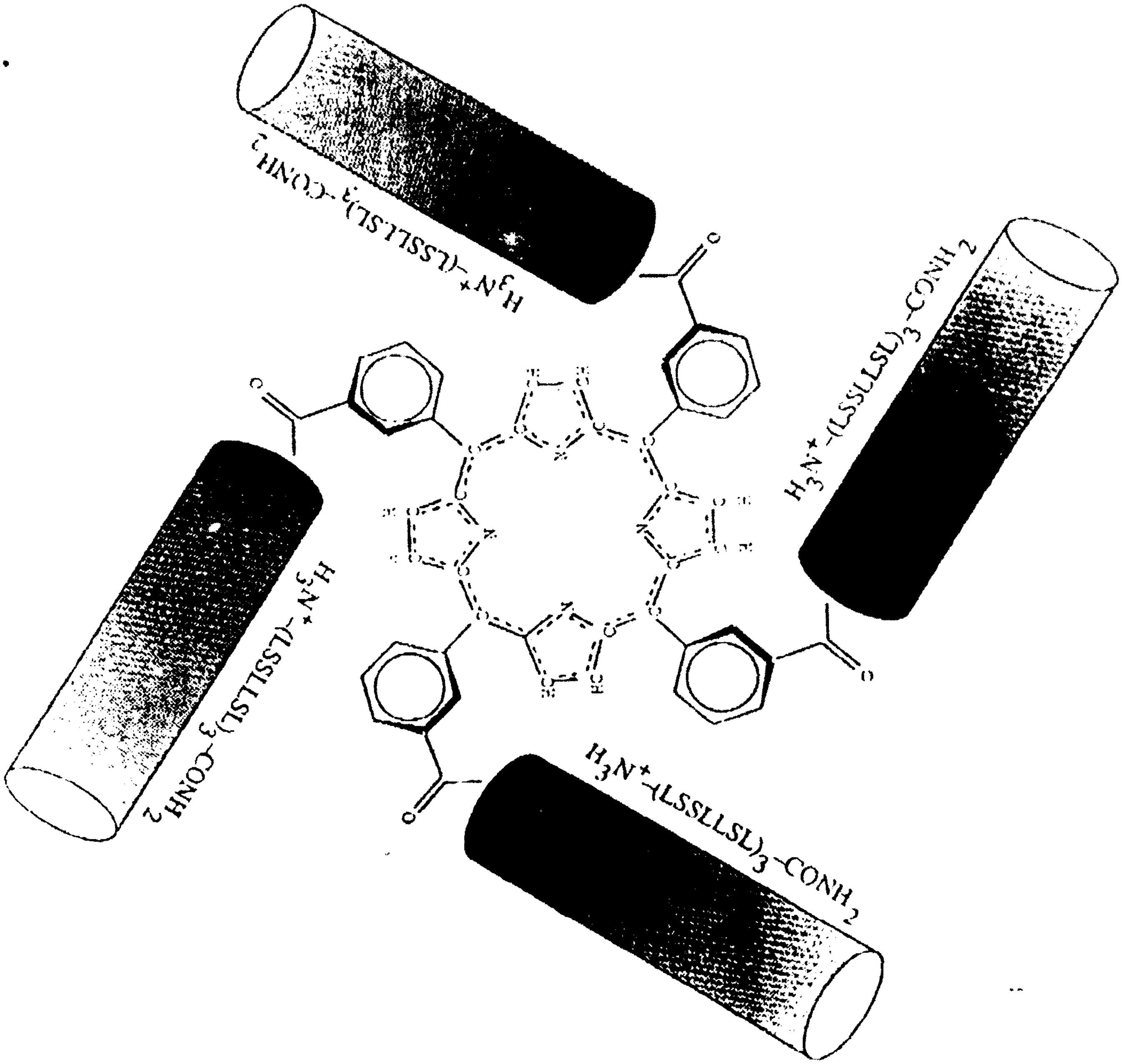
- Design of template assembled channels (pictured below)
- Control selectivity of permeant ions
- Design of selective ion-binding proteins.
- Design of models for metalloproteinases and redox proteins.

Accomplishments

- Characterization of Zinc-binding proteins
- The conformation and orientation of channel peptides in bilayers.
- Design of models for blue copper proteins.
- Synthesis of template-assembled channels.
- Modulation of channel lifetime.

Significance

- Learn to design proteins from scratch
- Learn how natural proteins work
- Design of Biosensors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